

1 **Effects of elevated CO₂ and temperature on rice brown planthopper, *Nilaparvata***
2 ***lugens* (Stål) populations in India**

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17 **Abstract**

18 Two populations of the brown planthopper (BPH), *Nilaparvata lugens* (Stål)
19 (Homoptera: Delphacidae) were collected from two hotspot locations of India –
20 Ludhiana (Punjab) in the north and West Godavari (Andhra Pradesh) in the south and
21 their biological parameters were studied under ambient conditions. Results showed that
22 the above two populations were notably different in three out of five biological
23 parameters recorded. The response of these two populations to climate variables *viz.*,
24 elevated temperature and increased levels of CO₂ was assessed. These conditions

25 prolonged the nymphal duration (14.2 days) and lowered female longevity (9.6 days),
26 fecundity (155.5 eggs/ ♀) and nymphal feeding rate (14.3 mm²) as compared to ambient
27 CO₂ and temperature across the populations. Honeydew excretion by adults was
28 significantly higher at elevated CO₂ than at the ambient level. At elevated CO₂ and
29 higher temperature, Ludhiana population recorded significantly longer nymphal
30 duration (15.9 days) and decreased amount of honeydew excretion by nymphs (13.8
31 mm²) as compared to the parameters recorded at elevated CO₂ and ambient temperature
32 (12.8 days and 32.8 mm², respectively). In contrast, West Godavari population recorded
33 significantly reduced female longevity and fecundity under elevated CO₂ and higher
34 temperature. Elevated CO₂ *per se* did not adversely affect BPH biology across
35 populations but with the concomitant increase in temperature, populations showed
36 varying response. Location specific mitigation strategies for management of hoppers
37 will be required to address the varying responses of populations to climate variables.

38 **Key words:** BPH, Fecundity, Honeydew, Ludhiana, West Godavari.

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42 **Introduction**

43 Global climate change is no longer for prediction but a reality. The global average
44 temperature which has now increased by 0.6°C is projected to further increase by 1.4 -
45 7.5°C; whereas the elevated atmospheric CO₂ level at 380 ppm presently is expected to
46 reach 560 ppm by the end of 21st century¹. It is also predicted that temperature increase
47 of about 2 to 3°C would result in 5 to 10% yield loss in the rice crop, if no proper
48 counter measure was adopted between 2030 and 2050². Climate change is expected to
49 have great impacts on crop pests directly as well as through their host plants indirectly³.
50 Many species of herbivorous insects tend to show altered behaviour under CO₂
51 enrichment and has been studied extensively in many insects^{4,5,6,7,8}. The consequences
52 differ among species and include retarded growth rate, increased nymphal development
53 time, higher mortality rates,^{9,10} accelerated development of eggs and larvae and delayed
54 emergence of adults¹¹. The brown planthopper (BPH), *Nilaparvata lugens* (Stål)
55 (Homoptera: Delphacidae) is one of the most serious pests of rice in both temperate and
56 tropical regions of East and South Asia and has reached outbreak levels over the past
57 few years¹². It is known for its seasonal migration and 'r' strategy pattern of life. It
58 directly damages the plant by sucking phloem sap, causing hopper burn, and also by
59 transmitting viral diseases¹³. It has emerged as the major pest of rice in India only after
60 1971, with cultivation of short statured high yielding and nitrogen-responsive varieties.
61 Widespread outbreaks of brown planthopper causing heavy yield losses were observed
62 in recent years^{14,15}. In 2005-2006, more than 485,000 hectares of rice in
63 southern Vietnam were severely affected by viral diseases spread by BPH, resulting in
64 losses valued at US\$120 million¹⁶. The planthopper populations from different
65 countries and areas within countries differ in their responses to rice varieties with the

66 same resistance genes^{17,18}. Similar differences may be reflected in their response to
67 climate change. There is scant information available on the impact of elevated CO₂ and
68 temperature individually and in combination on BPH and the variations in response of
69 populations from different geographical regions. Hence, the present study was
70 envisaged to assess the interactive effects of elevated CO₂ and temperature on BPH
71 populations collected from two different geographical regions of India which are
72 hotspots for the outbreak of this insect.

73 **Materials and methods**

74 **CO₂ chambers**

75 A specially designed closed climate controlled chamber was constructed with a
76 total dimension of 10'x8'x10'. It had three chambers each of 2.5'x8'. The chambers
77 were maintained at three levels of CO₂ concentration and temperature combinations,
78 continuously, at the ICAR-Indian Institute of Rice Research, Hyderabad (Latitude-
79 17°10"N, Longitude-78°E, Altitude -542m) as follows.

- 80 1. Ambient CO₂@ 380±25ppm and ambient temperature (aCO₂+aT),
- 81 2. Elevated CO₂@550±25ppm and ambient temperature (eCO₂+aT) and
- 82 3. Elevated CO₂@550±25ppm and elevated temperature (eCO₂+eT).

83 Fully automatic control and monitoring system with CO₂ sensors, Programmable Logic
84 Controller (PLC) and Supervisory control and data acquisition (SCADA) programme to
85 monitor the desired CO₂ level within the chambers, was established in an anteroom and
86 the climate variables as listed above were maintained throughout the study period. CO₂,
87 temperature and humidity were recorded continuously during the experiment.
88 Temperatures were 31°C/26°C for day/night cycle and relative humidity 70-80% for

89 ambient temperature and 34°C/29°C for elevated temperature and relative humidity was
90 60-70%.

91 **Insect material**

92 BPH populations were collected from two different states of the country. One
93 population was collected from Ludhiana of Punjab state, falling under hot semi arid
94 climatic conditions representing north-west India where rice crop is grown only during
95 June to November (Wet season). The second population was collected from West
96 Godavari District of Andhra Pradesh state which falls under hot sub humid to semi arid
97 conditions, where rice is grown in two seasons (Dry and Wet). The populations were
98 individually reared on young rice seedlings of susceptible cultivar TN1, using modified
99 Japanese method^{19,20} in flexi cages to avoid mating and intermingling of the two
100 populations in a green house at the Indian Institute of Rice Research, Hyderabad, India.

101 **Biological parameters**

102 **Baseline biological data of populations**

103 The biological parameters such as nymphal survival, nymphal duration,
104 longevity and fecundity of the two populations were studied in the laboratory under
105 ambient conditions. Newly emerged first instar nymphs were used for this experiment.
106 A single forty- five days old rice plant (*cv* TN1) was taken in a glass test tube
107 (25x200mm *dia*) containing Hoagland's nutrition media up to 5cm depth and twenty
108 nymphs were released into it. When plants showed yellowing due to feeding it was
109 replaced with plants of the same age. The level of Hoagland solution was maintained
110 by topping regularly. Observations were recorded on various biological parameters.

111

112 **Nymphal duration**

113 Five pairs of adults from each population were collected from the stock culture
114 and reared in CO₂ chambers designated as (F₀) generation; subsequently the newly
115 hatched nymphs of F₁ generation were transferred singly on 30 days old TN1 plant
116 placed in Hoagland solution inside a glass test tube (25x200mm). Forty replications
117 were maintained in each chamber. The rice seedling was replaced every alternate day.
118 Nymphal duration was calculated from the day of hatching up to adult emergence.

119

120 **Fecundity and longevity of adults**

121 One pair of newly emerged adults of F₁ generation was released on potted plant
122 of TN1 (45 days old), covered with a mylar cage. Every 3rd day, adults were transferred
123 to fresh 45 days old TN1 plants until the death of adults. Observations were also
124 recorded on nymphal hatching every day until no nymphal hatching was observed for
125 four consecutive days. Survival of the male and female hoppers was recorded and
126 longevity was calculated as the period from emergence to death.

127

128 **Honeydew excretion test**

129 Feeding behavior of the two populations at three different treatments was
130 estimated by honeydew excretion of third instars nymphs and newly emerged
131 brachypterous females individually as per protocol²¹. Five third instar nymphs or a one
132 day old brachypterous female starved for 2h, were released on 30 days old rice
133 seedlings (*cv* TN1) at the three leaf stage. Each treatment was replicated thrice and the
134 area of honeydew excretion was measured from the scanned image of the filter paper
135 using *Image J* software.

136 **Data analysis**

137 Data collected for each variable were analyzed by a General Linear Model
138 (GLM) SYSTAT 12 (Systat software Inc. Chicago IL) with CO₂ concentration and
139 temperature included as factors. Datasets were transformed where necessary to satisfy
140 assumptions of normality and homogeneity of variances. Where a significant interaction
141 between the two factors occurred in the GLM analysis, a *post hoc* comparison of means
142 was carried out. For datasets with homogenous variances, LSD test was used. Where
143 heteroskedasticity occurred that could not be removed *via* data transformation, the
144 Games and Howell method was used to compare means.

145 **Results**

146 **Baseline biological data of populations**

147 The two populations showed significant differences for various biological
148 parameters. Lower nymphal survival (48.5 %) was observed in West Godavari
149 population as compared to ninety per cent in Ludhiana population (Table 1). The latter
150 showed prolonged nymphal duration in brachypterous female and male and
151 macropterous male as compared to West Godavari population. Irrespective of wing
152 morphs, female and male hoppers took longer to develop in the Ludhiana population
153 (Table 1). Similar trend was observed in brachypterous and macropterous forms
154 irrespective of gender. The development of macropterous female forms was very low in
155 the study as food was not a constraint. The longevity of brachypterous female and male
156 was significantly higher in West Godavari population. On the other hand, the fecundity
157 was higher in Ludhiana population (116eggs/♀) as compared to West Godavari
158 population (62.5eggs/♀).

159 **Nymphal development**

160 The nymphal duration did not differ significantly in West Godavari population
161 among the treatments. But in Ludhiana population it was significantly prolonged in
162 eCO₂+eT (15.9 days) when compared to other treatments. Across the treatments,
163 nymphal duration was highest in eCO₂+eT (14.2 days). Across the populations, the
164 population from West Godavari had lesser developmental duration (12.8 days) than that
165 of Ludhiana (13.8 days) (Figure1& Table2).

166

167 **Longevity of adults**

168 In the West Godavari population, female longevity did not differ significantly
169 among the CO₂ treatments (Figure 2a & Table 2). On the other hand, the female
170 longevity in Ludhiana population was significantly lesser in eCO₂+eT (10.4 days) than
171 aCO₂+aT (15.0 days). Across the treatments, female longevity was significantly reduced
172 at eCO₂+eT (9.6 days) compared to other treatments. Across the populations, female
173 longevity was significantly higher in Ludhiana population (12.3 days) than in West
174 Godavari population (10.3 days). However, male longevity did not differ significantly
175 across the treatments and populations (Figure 2b & Table 2).

176

177 **Fecundity of female adults**

178 Fecundity increased in West Godavari population, at eCO₂+aT (275.5eggs/♀)
179 compared to eCO₂+eT (134.2eggs/♀) and aCO₂+aT (113.2eggs/♀) whereas it was not
180 significantly different in the Ludhiana population. Across the treatments, the fecundity
181 was significantly higher at eCO₂+aT (251.3eggs/♀) compared to eCO₂+eT

182 (155.5eggs/♀) and aCO₂+aT (188.3eggs/♀). However, across the populations, the two
183 did not differ significantly (Figure 3 & Table 2).

184

185 **Feeding assessed by honey dew secretion**

186 Honeydew excretion by third instar nymphs of West Godavari population was
187 on par in all the treatments. Ludhiana population showed significant difference between
188 eCO₂ treatments: lower amount of honeydew was excreted by third instar nymphs at
189 eCO₂+eT (13.8 mm²) compared to eCO₂+aT (32.8 mm²). Similarly, across the
190 treatments, significantly lower amount of honeydew was excreted at eCO₂ +eT (14.3
191 mm²) compared to eCO₂+aT (29.6 mm²) and aCO₂+aT (21.8 mm²) though the
192 difference among the populations was not significant (Figure 4a & Table 2).

193 Honeydew excreted by the brachypterous females showed significant differences
194 in West Godavari population, the feeding under eCO₂ treatments were on par, but lower
195 amount of honeydew was excreted by the adults under aCO₂+aT (166.3 mm²) compared
196 to other treatments (Figure 4b). In Ludhiana population, though higher amount of
197 honeydew was excreted by female adults under eCO₂+aT (323.0 mm²), it was not
198 statistically significant. Across the treatments, honeydew excretion was significantly
199 higher under eCO₂+aT (284.0 mm²) compared to aCO₂+aT (206.9 mm²) but
200 temperature fluctuations did not have a significant influence. Across the populations,
201 higher amount of honeydew excretion was noticed in Ludhiana population (274.4 mm²)
202 than in West Godavari population (213.3 mm²) (Figure 4b & Table 2).

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206 Discussion

207 Climate change is associated with warming, elevated CO₂ and regionally changed
208 precipitation²². Temperature directly influences the survival, development and
209 abundance of insects profoundly. Insects inhabiting the colder climates with marked
210 seasons have better tolerance to thermal extremes. Global warming may benefit such
211 insects which are currently exposed to cooler temperatures that are lesser than their
212 optima²³. Insects inhabiting the tropical regions are already living at environmental
213 temperatures close to their optimum and any further increase may have adverse effects.

214 Environmental heterogeneity affects the phenotypic expressions of insects²³.
215 Similarly, biological evidence suggests that rice planthoppers show significant
216 geographic structure¹⁹. Our studies too indicate geographical variations exist in
217 planthoppers with respect to biological parameters *viz.*, survival, developmental period,
218 longevity, fecundity and wing morphs.

219 When geographical variations exist in populations without climate stress, the
220 response to climatic variables may also differ. In our results, elevated temperature with
221 increased levels of CO₂, significantly affected the biological parameters of BPH. It
222 prolonged the nymphal duration and lowered longevity, fecundity and nymphal feeding
223 rate. When the temperatures were lower than the optimum range or above optimum
224 range, it was observed that biological functions like feeding, rate of development and
225 longevity were lowered while nymphal duration was prolonged in BPH²⁴. Our results
226 are in line with earlier findings²⁵ that the duration of nymphal growth was shorter at a
227 mean temperature of 30°C, and prolonged with increased temperature. Other studies
228 have reported that temperature between 25° and 30°C was optimal for egg and nymphal

229 development of BPH^{26,27,28,29,30} whereas temperatures above 30°C, was found to be
230 unfavourable for insect survival^{27,28,30,31}.

231 Female longevity was significantly reduced under elevated CO₂ and elevated
232 temperature in Ludhiana population. A similar trend was observed in West Godavari
233 population though not statistically significant. However, male longevity did not show
234 any significant difference in both populations. The yellow sugarcane aphid, *Sipha flava*
235 showed shorter longevity at elevated CO₂ (500 ppm) and elevated temperature (28°C)
236 than at the same CO₂ conditions but lower fluctuating temperatures³². The female
237 longevity of *S. flava* was also found to be shorter at elevated CO₂ (700 ppm) and
238 elevated temperature (28°C) than ambient CO₂ and temperature³².

239 We observed that the fecundity of BPH increased at elevated CO₂ levels but,
240 elevated temperature decreased the fecundity of BPH across the treatments. The number
241 of eggs laid by BPH was found to be lower, at higher temperature regimes of 34.0 and
242 36.0°C and observed that these increased temperatures were not suitable for egg laying
243 and development of BPH³³. The number of eggs laid by BPH decreased rapidly as the
244 temperature increased³⁴. However, in contrast it was reported that fecundity was higher
245 at elevated temperature with elevated CO₂ than at ambient temperature with ambient
246 CO₂³⁵. This study was however conducted in a temperate region which has been
247 hypothesized to benefit insects that are exposed to cooler temperatures³⁶. The elevated
248 temperature in our case, being in a tropical region, was a +3°C increase from 31/26 to
249 34/29°C (day/night cycle) which might have lead to decreased egg laying capacity of
250 BPH. Further, it was observed that fecundity of BPH showed varying results between
251 populations with respect to elevated temperature and CO₂. Population from West
252 Godavari which is a coastal region showed decreased rate of fecundity at elevated

253 temperature and elevated CO₂ whereas the Ludhiana population exposed to distinct hot
254 and cooler climates, was not influenced by elevated temperature and elevated CO₂. The
255 optimum temperature for survival and reproduction varies between insect species and
256 populations of each species²². Our results indicate that the feeding rate of third instar
257 nymphs was more influenced by the combined effect of elevated CO₂ and temperature
258 in both the populations, which was in accordance with earlier reports^{5,8} that elevated
259 temperature may directly reduce feeding in insects or the reduced water content in
260 stem parts and altered osmotic potential in the phloem-sap may reduce the feeding rate.
261 In case of adults, elevated CO₂ increased feeding in both the populations, while
262 temperature did not have a significant impact. On the other hand, earlier literature states
263 that high temperature exposure reduced feeding activity and honeydew production in
264 both life stages (nymphs and adults) of *N. lugens*³⁷. This clearly indicates that insects
265 from tropical and temperate regions exposed to different temperature regimes will show
266 varied responses to climate change factors.

267 The average temperature range prevailing at Ludhiana is wider with a minimum
268 temperature being 9.5°C and maximum of 35.3°C while the temperature range at West
269 Godavari is narrower with minimum temperature of 25.3°C and a maximum of 31.8°C
270 (mean of two years 2013-2014). Across all the parameters, Ludhiana population
271 responded positively to climate change with prolonged nymphal duration, higher
272 longevity of females and higher feeding rate. The results of the study indicated that
273 population exposed to a greater temperature range *i.e.* lower/higher temperatures may
274 be positively impacted.

275 The present study revealed that elevated CO₂ (550 ppm) with elevated
276 temperature (+3°C) significantly increased nymphal duration but reduced female

277 longevity, fecundity and amount of honeydew excreted by nymphs of brown
278 planthopper in comparison with those noted at elevated CO₂ and ambient temperature
279 across the two populations studied. However, at elevated CO₂ and ambient temperature,
280 significantly higher amounts of honeydew excretion by adults as compared with those at
281 ambient CO₂ and ambient temperature were recorded. Performance of BPH populations
282 from Ludhiana and west Godavari was notably different in three out of five parameters
283 recorded indicating that populations have a differential response to environmental
284 changes. Future studies should focus more on studying responses of biotypes and
285 populations to projected changes in the environment and formulate risk management
286 strategies specific to each region.

287

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398 **Table 1. Biological parameters of two populations of brown planthopper, *N. lugens***

Parameters	West Godavari (Mean±S.E)	Ludhiana (Mean±S.E)	t value (df)	P-value
Nymphal Survival (%)	48.5±1.5	90.0±0.5	5.36 (18)	0.000
Nymphal Developmental Duration (days)				
Brachypterous (male+female)	13.6±0.2	19.9±0.5	8.42 (104)	0.000
Macropterous (male+female)	12.9±0.2	18.2±1.0	5.26 (25)	0.000
Female (Brachypterous+macropterous)	13.5±0.3	20.8±1.3	7.39 (64)	0.000
Male (Brachypterous+macropterous)	13.3±0.3	18.1±1.2	6.77 (66)	0.000
Brachypterous female	13.6±0.3	21.0±1.3	7.00 (60)	0.000
Brachypterous Male	13.5±0.3	17.8±1.2	4.59 (42)	0.000
Macropterous Male	12.9±0.2	18.6±1.1	5.32 (22)	0.000
Adult Longevity (days)				
Brachypterous Female	15.8±0.7	12.5±0.9	-2.35 (13)	0.030
Brachypterous Male	10.0±1.0	6.1±0.6	-2.90 (8)	0.020
Macropterous Male	6.6±0.8	7.5±0.6	0.83 (9)	0.428
Fecundity (No. of eggs laid/female)				
Brachypterous Female	62.5±7.5	116.4±9.2	-4.40 (11)	0.001

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401 **Table 2. Summary of GLM analysis (ANOVA) results for effect of elevated CO₂**402 **and temperature on brown planthopper, *N. lugens***

Measurements	Treatments	F-value (d.f.)	P-value
Nymphal duration	West Godavari population- CO ₂ and temperature	5.43(2)	0.01
	Ludhiana population- CO ₂ and temperature	35.01(2)	0.00
	CO ₂ and temperature (Across treatments)	13.85(2)	0.00
	Population (Across populations)	5.46(1)	0.00
Longevity female	West Godavari population- CO ₂ and temperature	2.98(2)	0.06
	Ludhiana population- CO ₂ and temperature	3.30(2)	0.04
	CO ₂ and temperature (Across treatments)	4.11(2)	0.02
	Population (Across populations)	4.89(1)	0.03
Longevity male	West Godavari population- CO ₂ and temperature	2.16(2)	0.13
	Ludhiana population- CO ₂ and temperature	0.88(2)	0.42
	CO ₂ and temperature (Across treatments)	0.07(2)	0.93
	Population (Across populations)	3.67(1)	0.06
Fecundity	West Godavari population- CO ₂ and temperature	16.71(2)	0.00
	Ludhiana population- CO ₂ and temperature	2.53(2)	0.10
	CO ₂ and temperature (Across treatments)	3.23(2)	0.03
	Population (Across populations)	0.71(1)	0.40
Honeydew excretion by third instar	West Godavari population- CO ₂ and temperature	2.17(2)	0.15
	Ludhiana population- CO ₂ and temperature	4.07(2)	0.04
	CO ₂ and temperature (Across treatments)	6.58(2)	0.01
	Population (Across populations)	0.09(1)	0.76
Honeydew excretion by female adults	West Godavari population- CO ₂ and temperature	9.82(2)	0.01
	Ludhiana population- CO ₂ and temperature	3.59(2)	0.09
	CO ₂ and temperature (Across treatments)	3.40(2)	0.05
	Population (Across populations)	7.98(1)	0.01

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405 **Figure 1.** n=40 replications for each treatment; aCO₂+aT- ambient CO₂ and ambient
406 temperature; eCO₂+aT- elevated CO₂ and ambient temperature; eCO₂+eT- elevated CO₂
407 and elevated temperature. Different letters on the bars in a panel denotes significant
408 difference (LSD test, p=0.05).

409 **Figure 2a.** n=10-12 for each treatment.

410 **Figure 2b.** n=10-12 for each treatment

411 **Figure 3.** n= 5-10 for each treatment

412 **Figure 4a.** n= 5 for each treatment

413 **Figure 4b.** n= 5 for each treatment.

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428 **Figure 1. Nymphal duration:** Effects of CO₂ concentration and temperature on
429 nymphal duration of the brown planthopper, *N. lugens* on TN1 variety.

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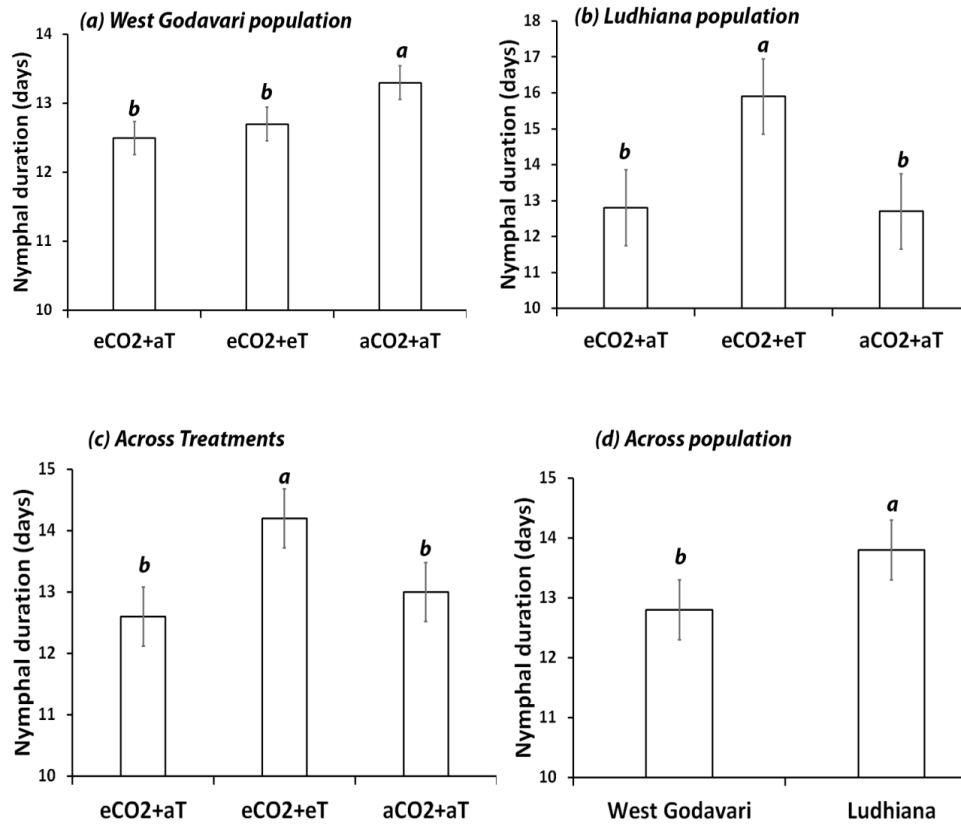
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451 **Figure 2a. Female Longevity:** Effects of CO₂ concentration and temperature on
452 female longevity of the brown planthopper, *N. lugens* on TN1 variety.

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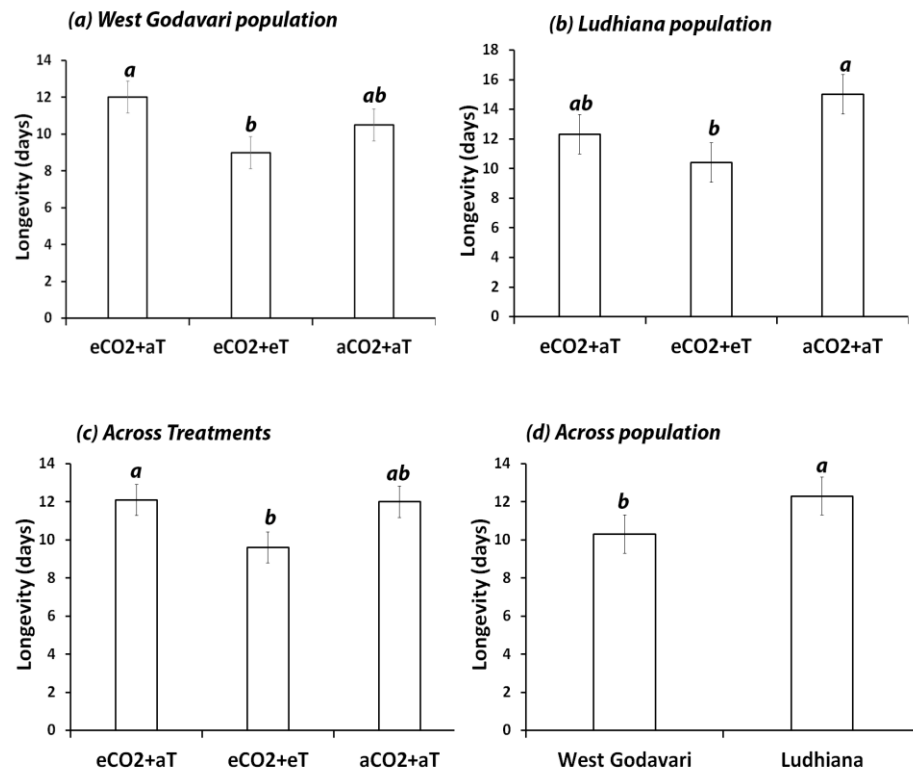
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464 **Figure 2b. Male Longevity:** Effects of CO₂ concentration and temperature on male
465 longevity of the brown planthopper, *N. lugens* on TN1 variety.

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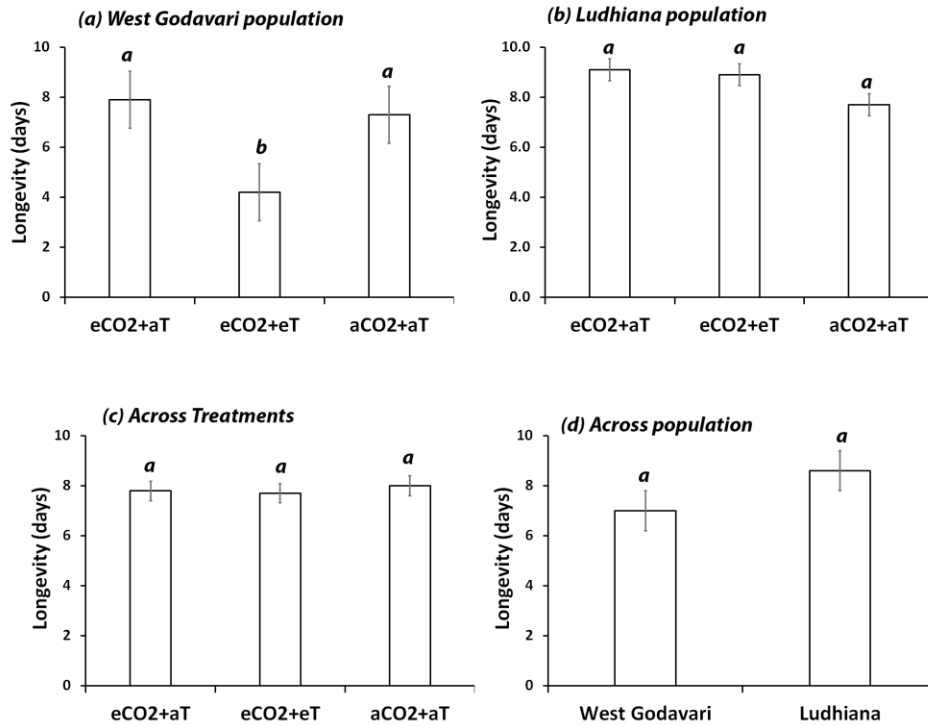
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478 **Figure 3. Fecundity of female adults:** Effects of CO₂ concentration and temperature
 479 on fecundity of the brown planthopper, *N. lugens* on TN1 variety.

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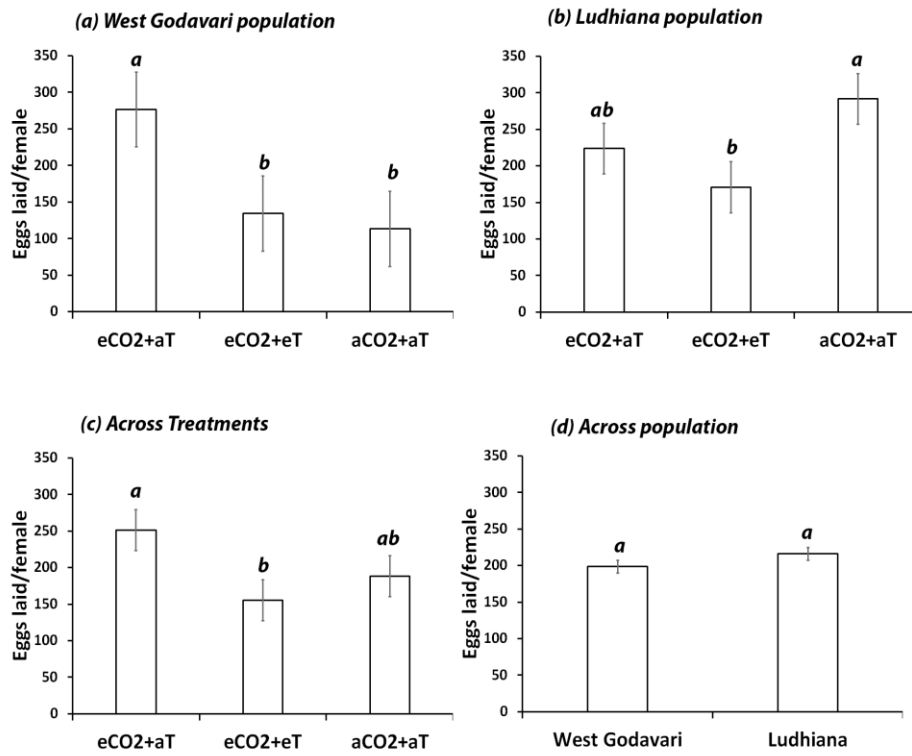
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492 **Figure 4a. Honeydew excretion by third instar nymphs: Effects of CO₂**
 493 concentration and temperature on honeydew excretion in 24hrs per five third instar
 494 nymphs of the brown planthopper, *N. lugens* on TN1 variety.

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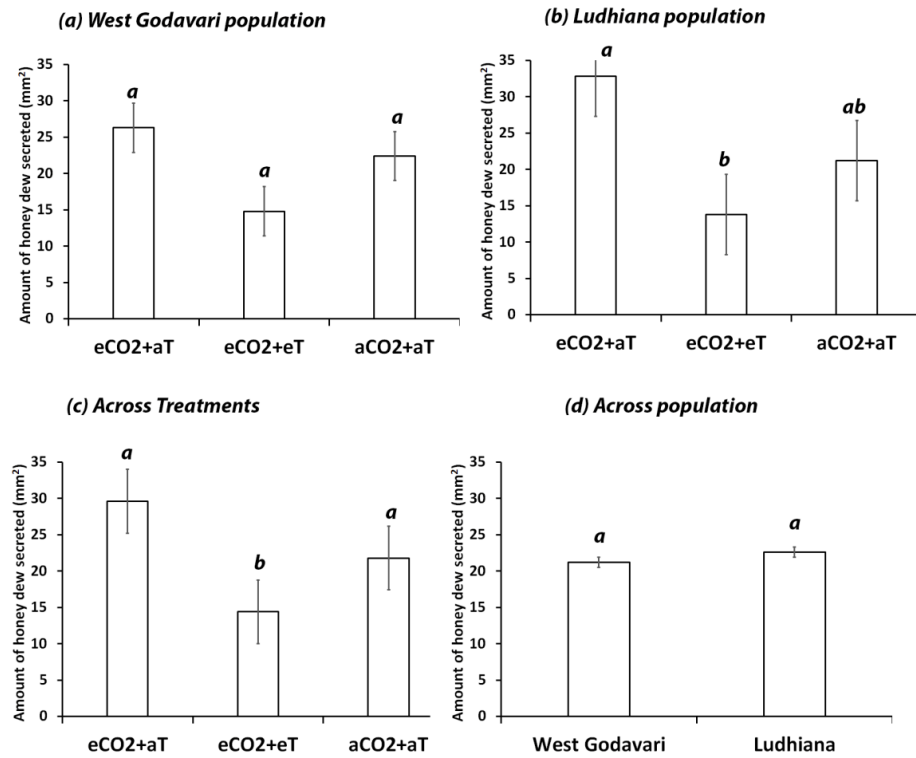
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516 **Figure 4b. Honeydew excretion by brachypterous female adults:** Effects of CO₂
 517 concentration and temperature on honeydew excretion in 24hrs per five brachypterous
 518 females of the brown planthopper, *N. lugens* on TN1 variety.

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